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October 23, 2003

Marianne L. Horinko, Acting Adminstrator
U.S. Environmental Protection Agency
Ariel Rios Building
Room 3000, #1101-A
1200 Pennsylvania Ave., N.W.
Washington, DC 20460

Subject: Comments on the HPV Test Plan for Carbamate hydrochloride

Dear Acting Administrator Horinko:

The following comments on DuPont's test plan for the chemical Carbamate hydrochloride are submitted on behalf of the Physicians Committee for Responsible Medicine, People for the Ethical Treatment of Animals, the Humane Society of the United States, the Doris Day Animal League, and Earth Island Institute. These health, animal protection, and environmental organizations have a combined membership of more than ten million Americans.

E. I. duPont de Nemours & Company, Inc. submitted its test plan on December 17, 2002 for the chemical Carbamate hydrochloride (CAS No. 65206-90-8), or F3455.HCl, which exists as a solid but is transported in bulk as the hydrochloride salt in water. F3455.HCl is consumed by chemical reaction and is not present in the distributed product. The commercially available product (DuPont does not provide the name of this product) contains 35-51% F3455.HCl. We concur with DuPont that this chemical classifies as a closed system intermediate, eliminating the need for repeated dose and reproductive toxicity testing in the SIDS battery.

At this time, however, we would like to point out that this test plan appears incomplete and lacks significant detail and we feel that efforts to minimize animal testing are not being taken seriously. DuPont's proposal to conduct an acute fish toxicity test (OECD 203) and a developmental study in rats (OECD 414) will result in the death of at least 1,400 animals.

There is very limited toxicity data available for F3455.HCl and, therefore, DuPont used structure activity relationship programs and models, specifically ECOSAR, to estimate toxicity to fish and other aquatic organisms. We commend this approach for estimating ecotoxicity; the EPA has also encouraged the use of this method (EPA 2002). F3455.HCl has a very large LC50 value (11,334 mg/L using ECOSAR), making an acute hazard in an aquatic environment highly unlikely for this chemical. Considering the limited

exposure potential of F3455.HCl and its classification as a closed system intermediate, it is completely unwarranted to conduct further, unreliable animal tests, which would kill many fish and only serve as a "check-the-box" exercise.

If DuPont wishes to further investigate the acute fish toxicity of F3455.HCl, we urge it to use an *in vitro* method. TETRATOX, an assay based on the *protozoan Tetrahymena pyriformis* (Larsen 1997), is the most appropriate. With 50% growth impairment as the endpoint, the results of this assay show close similarity to toxicity in the fathead minnow (Schultz 1997). The extensive available information demonstrates that TETRATOX is an effective alternative to fish testing. It is in fact already used extensively in industry, and is being considered for regulatory acceptance by the OECD. It is also rapid, easy to use, and inexpensive. On October 23, 2001, PETA and PCRM held a meeting with EPA to facilitate incorporation of an *in vitro* aquatic toxicity test into the HPV program, and Dr. Schultz (Professor of Predictive Toxicology, University of Tennessee College of Veterinary Medicine) made a presentation about TETRATOX. On December 5, 2001, PCRM scientist Nicole Cardello presented the details of this meeting, and our proposal, in a letter to EPA Assistant Administrator Stephen Johnson. After almost two years, there has still been no response from Mr. Johnson or anyone else in the agency. We again request a thoughtful, scientific and specific reply to this letter. It is the stated goal of the EPA to incorporate *in vitro* methods into the HPV program, and this presents an ideal opportunity for action rather than words by DuPont.

The recently validated *Dar*T test is another prospective replacement for *in vivo* studies. The test protocol and performance parameters are described in detail in Schulte (1994) and Nagel (1998). Briefly, however, the *Dar*T test uses fertilized zebrafish (*Danio rerio*) eggs as a surrogate for living fish. The exposure period is 48 hours, and assessed endpoints include coagulation, blastula development, gastrulation, termination of gastrulation, development of somites, movement, tail extension, eye development, circulation, heart rate, pigmentation and edema. Endpoints comparable to *in vivo* lethality include failure to complete gastrulation after 12 hours, absence of somites after 16 hours, absence of heartbeat after 48 hours, and coagulated eggs. The other endpoints provide further insight for a more detailed assessment of test substances. The reliability and relevance of the *Dar*T test have recently been confirmed in an international validation study coordinated and financed by the German Environmental Protection Agency, and predictions of acute toxicity from the *Dar*T test were highly concordant with *in vivo* reference data (Schulte 1996). This *in vitro* test has been accepted in Germany as a replacement for the use of fish in the assessment of wastewater effluent (Friccius 1995), and is clearly suitable for immediate use as a replacement for the use of fish in the HPV program's screening-level toxicity studies.

With respect to conducting OECD 414, it is alarming that DuPont proposes a developmental toxicity test that will kill 1,300 animals, when the combined reproduction/developmental screen, OEDC 421, will reduce animal deaths by half and is adequate for a screening level program such as HPV. The EPA should require justification from DuPont as to why the OECD 414 is planned. Furthermore, an *in vitro* embryotoxicity test method, the rodent embryonic stem cell test, has been validated by

the European Centre for the Validation of Alternative Methods, and the Centre's Scientific Advisory Committee has concluded that this test is ready to be considered for regulatory purposes (Genschow 2002). If a positive result is found in the embryonic stem cell test, F3455.HCl should be treated as a development toxicant/teratogen, and no further testing should then be carried out within the HPV program. We strongly urge DuPont to consider this *in vitro* method, as have other submitters in the HPV program, in order to spare large numbers of animals and hope that DuPont will contact us for advice about laboratories in the U.S. that are currently conducting this test. This test is particularly appropriate for use in this case of a closed system intermediate with little or no potential exposure. Although we have written to the EPA repeatedly concerning the inclusion of the embryonic stem cell test in the HPV Program, with correspondence dating back more than six months, we have received no detailed reply. This would be a great opportunity for DuPont to work with EPA and the animal welfare community to incorporate this validated non-animal test into the HPV program.

Lastly, we are concerned that little attempt has been made to categorize F3455.HCl with similar compounds. Specifically, DuPont did not specify any chemically similar compounds to F3455.HCl in its test plan, only mentioning that ECOSAR predictions for this chemical were based on toxicity data for "classes of compounds with similar modes of action" (Robust Summary, pg.1). We recommend that DuPont identify the compounds that can be expected to be of similar toxicity to F3455.HCl as data for similar chemicals may be used to bridge data gaps for developmental toxicity as well as other toxicological endpoints in the SIDS battery.

Based on these considerations, we request that EPA defer comments on DuPont's proposal and incorporate the above revisions into a new and improved test plan. Thank you for your attention to these comments. I can be reached at 202-686-2210, ext. 327 or by email at *meven@pcrm.org*.

Sincerely,

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Director of Toxicology Research

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